Molecular Detection and Environmental Survey of *Helicobacter pylori*

Project Scope

Helicobacter pylori is a ubiquitous gram-negative bacterium present in the gastrointestinal systems of more than half of the population worldwide. It is a leading cause of peptic ulcers and contributes to a variety of other illnesses, ranging from childhood malnutrition to gastric cancer. It is also known to increase susceptibility to other water- and food-borne pathogens. Although its primary mode(s) of its transmission remains unknown, some epidemiological data suggest contaminated water as a possible transmission route, especially in developing countries. In the environment and *in vitro*, *H. pylori* has been shown to transform from a culturable spiral-shaped morphologic form into a viable, but as yet nonculturable, coccoid form.

Recognizing the potential of *Helicobacter pylori* to be transmitted through drinking water, EPA placed it on the first (1998) and most recent (2005) Contaminant Candidate List (CCL). Due to the extreme difficulty of recovering *H. pylori* from water using routine culturing methods. EPA identified it as a contaminant for which improved analytical methods were necessary and identified the development of such a method as a research priority.

This objective of this research was to develop and validate an accurate and sensitive method for rapid detection of *H. pylori* (both the culturable and coccoid forms) from environmental waters. To achieve their research objective, the researchers investigated the use of the following molecular and immunological methods:

1. Proteomics (i.e., analyzing patterns of all expressed proteins) specific to each morphologic form of *H. pylori* under laboratory conditions;
2. Indirect fluorescent antibody (IFA)

Grant Title and Principal Investigator

Molecular Detection and Environmental Survey of Vegetative and Coccoid *Helicobacter pylori*
(EPA Grant #R828037)

Manoucher Shahamat, University of Maryland

Key Findings and Implications

Analytical Accomplishments:

- A proteomic-based detection method was successfully developed that demonstrated clear protein expression signature differences between culturable and coccoid (viable but not culturable) cells of *Helicobacter pylori* under laboratory conditions. However, the method was found to be highly time- and resource-intensive and not amenable for use with environmental water samples.
- Monoclonal antibodies for *H. pylori* were successfully developed for use with indirect fluorescent antibody (IFA; immunological-based) methods for its detection under laboratory conditions. However, the IFA method is not amenable for use with environmental water samples due to interference from particulate matter and other microorganisms.
- Two sensitive and complimentary polymerase chain reaction (PCR)-based methods for the detection of *H. pylori* were successfully developed and tested under laboratory conditions and using spiked environmental water (well and river) samples.

Implications of Research:

- The dual PCR detection system provides an effective, rapid, and accurate presumptive assay for determining the presence of *H. pylori* in cultures and in environmental water samples. They can also be used in conjunction to help identify potential sources of *H. pylori* in environmental waters.

Publications include 1 peer reviewed article and 3 conference presentations.

Project Period: May 2000 to May 2003

Relevance to ORD's *Drinking Water Research Multi-Year Plan* (2003 Edition)

This project contributes directly to the second of three Long-term Goals for drinking water research: (2) By 2010, develop new data, innovative tools and improved technologies to support decision making by the Office of Water on the Contaminant Candidate List and other regulatory issues, and implementation of rules by states, local authorities and water utilities.

Research under this grant supported the development of complimentary, accurate, and rapid detection methods for both the culturable and coccoid forms of *H. pylori* in environmental waters, including treated drinking water, groundwater, and surface waters. The combination of two complimentary, sensitive PCR-based methods developed in this research can also be used to help identify potential sources of this important waterborne pathogen.

methods in conjunction with monoclonal antibodies specific to *H. pylori* to identify vegetative and coccoid forms under laboratory conditions; and

3. Polymerase chain reaction (PCR)-based molecular methods for detection of *H. pylori* in the laboratory and from environmental samples.

The ability to detect both morphological forms is crucial for identifying and quantifying *H. pylori* occurrence in environmental water samples and improving understanding of its public health and ecological significance.

Project Results and Implications

Proteomics: The focus of the proteomic research was to identify unique protein expression signatures (PES) that could be used to distinguish between *Helicobacter* species, and among strains of *H. pylori* under laboratory conditions. The approach assessed under this grant was a method to distinguish between the vegetative and coccoid forms of *H. pylori*. Proteins were extracted using a variety of protease inhibitors, detergents, and nucleases, and separated according to their molecular weight and charge differences. The composite gels were fixed, stained, scanned, and imported for comparison of "protein spots" among strains and growth phases using an image analysis software program. Although the proteomic-based approach was able to demonstrate clear PES differences between culturable and coccoid cells, two major problems were encountered: (1) in order to process a sample of *Helicobacte*r a visible pellet of cells was needed, which would be very difficult to collect in practical applications because environmental concentrations are typically quite low compared to those used in the laboratory; and (2) the method was found to be extremely labor-intensive and required far more resources than originally anticipated.

Indirect Fluorescent Antibody: In this research, monoclonal antibodies for *H. pylori* were developed for use with IFA methods for its detection in a laboratory setting. Although the antibodies appeared to be specific for *H. pylori*, and an IFA approach appeared feasible for clinical (and clean water) samples, the researchers concluded that its use for environmental water samples containing other microorganisms and inert particulate matter would be problematic. For these reasons, no further research was conducted on proteomic- or IFA-based detection methods for *H. pylori* in environmental waters, and the remaining grant resources were focused on the development and validation of two PCR-based detection methods.

Polymerase Chain Reaction (PCR): Two sets of primers were developed for use in PCR amplification. The objective was to develop an accurate and rapid detection method for both morphological forms of *H. pylori* in cultures and filtered-sterilized environmental water samples. The latter were obtained from well

and river waters located throughout Maryland and subsequently spiked with various strains of *H. pylori*, other *Helicobacter* species, and other bacterial genera, species, and strains.

PCR Primers for the glmM Gene
A total of 26 strains of *H. pylori*, including both coccoid and helical forms, were tested using PCR primers for phosphoglucosamine mutase (*glm*M; formerly called the urease C or *ureC* gene). The results were positive for all *H. pylori* strains tested. Four other *Helicobacter* species were also tested using the *glmM* primers, with two species testing positive and two testing negative. Four strains of *Camplobacter jejuni* and 18 strains of other microbial species tested negative. Although PCR amplification of *glmM* can detect *H. pylori*, because it also detected a number of other *Helicobacter* species, the researchers concluded a more selective, complementary technique was needed to differentiate *H. pylori* from other *Helicobacter* species.

PCR of H. pylori Unique 16S rRNA Flanking Region
A genome sequence analysis by the researchers revealed that the 16S rRNA and 23S rRNA genes are not contiguous in the *H. pylori* chromosome as they are in all other *Helicobacter* species analyzed. In a series of experiments, selective PCR primer sets were developed and assessed to target the hypervariable flanking region upstream of the 16S rRNA gene for the specific detection of *H. pylori* (coccoid and helical forms). Using 32 strains of *H. pylori*, six strains of other *Helicobacter* species, eight strains of *Campylobacter jejuni*, and 21 strains belonging to several different bacterial genera, one PCR primer set was found to be highly specific for only *H. pylori* strains.

The two PCR-based methods for the detection of *H. pylori* developed during this research, one targeting the *glmM* gene and the other targeting the sequence upstream of the 16S rRNA gene, are complementary to each other. Whereas the *glmM*-specific PCR primers provide a rapid, sensitive presumptive assay for the presence of *H. pylori* and closely related *Helicobacter* species the primers for sequences flanking the 16S rRNA gene can confirm the presence of *H. pylori* and be subsequently used to help identify potential sources of this important pathogen in environmental waters. Although the dual PCR detection system does not distinguish viable from nonviable bacteria in any given sample, it provides an effective and rapid assay for determining the presence of *H. pylori* in cultures and in environmental water samples.

Investigators

M. Shahamat, University of Maryland
M. Alavi, University of Maryland
J. Gonzalez, University of Maryland
D. Maeder, University of Maryland
F. Robb, University of Maryland
K. Sowers, University of Maryland
J. Watts, University of Maryland

For More Information

NCER Project Abstract and Reports:

http://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/823

Peer Reviewed Publications

Shahamat, M., Alavi, M., Watts, J.E.M., Gonzalez, J.M., Sowers, K.R., Maeder, D.W., and Robb, F.T. 2004. Development of two PCR-based techniques for detecting helical and coccoid forms of *Helicobacter pylori*. Journal of Clinical Microbiology 42(8):3613-3619.